# Effect of Replacement of Soybean Meal with Duckweed (*Lemna minor*) Meal on the Growth Performance and Feed Utilization in Nile Tilapia Fingerlings

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**Abstract:** The present study was designed to evaluate the use of Duckweed (*Lemna minor*) as are placement of soybean meal Protein for Nile tilapia (*Oreochromis niloticus*) fingerlings. Duckweed was incorporated into four isonitrogenous (30% crude protein) and isocaloric diets (gross energy (448) Kcal/100g). The replacement levels were 25, 50, 75 and 100% of the soybean meal protein diet in addition to control diet. The experiment lasted for 56 days. The best final weight gain, specific growth rate, survival rate and feed utilization (FCR, FE and PER) were significantly ($p<0.05$) higher for group of fish fed on diet replaced with 25% Duckweed from Soybean protein diet. The highest feed conversion ratio was obtained for group of fish fed on diet replaced by 100% Duckweed. There was a significant difference ($p<0.05$) in protein and lipid in carcass proximate composition of the fish feed on diet replacement with 25% Duckweed from Soybean meal protein diet compared to those on the experimental groups. In conclusion, the study indicates that replacement soybean meal protein with 25% duckweed was the best in terms of growth performance and feed utilization.

***Keywords:*** Duckweed (*Lemna minor*)**,** replacement, growth performance, feed utilization, carcass composition *Nile tilapia***.**

## INTRODUCTION

The human demand of fish consumption is increased all over the world. Fish is about to become the main alternative source of animal protein. The culture trend of tilapia the exponential growth of the aquaculture sector during the past two decades is a result of the progressive intensification of production systems and use of quality feeds, which meet the nutritional requirements of cultured fish (FAO, 2012). Tilapia (*Oreochromis niloticus*), is widely cultured in the world. They tolerate a wide range of water quality conditions and could develop resistant to many diseases. Tilapia is a hardy prolific, fast growing tropical fish, and it can survive on a diversity of food (El-Sayed, 2006). Tilapia aquaculture represents about 42% of world total aquaculture fish production, and about 64% of the total fish production in Egypt (GAFRD, 2011). Demand and cost of fish feed and the global fishmeal price has increased more than two fold in recent years (FAO, 2013). One of the biggest problems facing the utilization of fish nutrition, in many aquaculture operations today, feed accounts more than half of the variable operating cost (NRC, 2011).

Therefore, the potential use of unconventional foodstuffs such as algae, for substitution the high cost ingredients such as fishmeal is very important. Algae have attention as a possible alternative protein source for cultured fish, particular in tropical and subtropical developing countries where algae production rates are high and their higher protein, vitamins and essential fatty acids contents (El-Hindawy *et al.,* 2006). Duckweed (*Lemna minor*) is a cyanobacterium that has been commercially cultivated for more than 10 years due to its high nutritional content; e.g. protein, amino acids, vitamins, minerals, essential fatty acid and b-carotene (Mohedano, 2009).

Nowadays, Duckweed meal has been known for its high nutritive value with as much as 40% and above crude protein depending on the culture system (Hassan and Edward, 1992). Many studies have explored alternative dietary protein sources for tilapia culture (Nguyen *et al.,* 2009; Zhao *et al*., 2009). Studies have been conducted on the use of aquatic plants in tilapia feeds (El-Sayed, 1994). Duckweeds are good food for tilapia, as it contains about 35%–45% crude protein, good amino acid balance, and mineral profile (Mbagwu and Adeniji, 1990). Mohedano *et al*. (2005) reported a reduction in feeding costs when replacing fishmeal with duckweed meal in tilapia diets. Tavares *et al*. (2008) showed that pelletized duckweed can represent up to 50% of a tilapia diet without affecting on weight gain of tilapia fingerlings.

The present study was aimed to investigate the effect of replacement soybean protein with different levels of duckweed (0, 25, 50, 75 and 100%) on growth performance, feed utilization and economic analysis for monosex Nile tilapia fingerlings.

## MATERIALS AND METHODS

The experiment was represented at El-Salam private fish hatchery, El Kantara, Ismailia- Egypt. The experiment aim to investigate, the effect of replacement of soybean meal by duckweed at levels of 25, 50, 75 and 100% in addition to control diet of monosex (*O. niloticus*) fingerlings.

### Water quality parameters

Water temperature and dissolved oxygen were measured by mettle Toledo, model 128.s/No1242 respectively. Other water quality including pH and ammonia were measured every two days by pH meter (Orion model 720A, s/no 13062) and ammonia meter by Hanna ammonia meter. The averages of water quality parameters are presented in (Table 1).

### Experimental unit

Fish were stocked in 15 hapa (1.5 m × 1.00 m × 1.m) randomly divided into three equal experimental

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groups (50 fingerlings / replicate, three replicate hapas). The hapa were supplied all day with air blowers. Water temperature was maintained at (28-25°C) inside green house. The part of water hapa was exchanged with fresh water 10% /day.

**Experimental fish**

Seven hundred and fifty fingerling monosex Nile tilapia (*O. niloticus*) with average initial body weight of 4 ± 0.1 g were obtained from El-Salam Fish Hatchery, Kantra, Ismailia governorate, Egypt. Fish were homogenous in body weights and apparently healthy. Fish were acclimated to farm conditions for 2 weeks' prior before the experiment study.

**Experimental diets**

Five isonitrogenous and isocaloric diets were formulated from practical ingredients (Table 2) where the control diet without duckweed and the other four diets were replacement of soybean meal protein with duckweed at levels 25, 50, 75 and 100% respectively. The experimental diets were formulated to contain almost 30% crude protein and gross energy 444 Kcal/100 g. The experimental diets were prepared by individually weighing of each component thoroughly mixing the mineral, vitamins and additives with corn. This mixture was added to the components together with oil. Water was added until the mixture became suitable for making granules. The wet mixture was passed through CBM granule machine with powders. The produced were dried at room temperature then kept until experimental start. The composition and proximate analysis of the experimental diets are presented in Table (2). The fish were hand-fed at rate 5% 4 times/day (7, 9, 11, 1 and 3 pm) throughout the experimental period 56 days.

**Table (1):** Water quality parameters

| Experimental | Temperature | Dissolved Oxygen | Ammonia | pH |
|---|---|---|---|---|
| **Parameters** | 28-25 | 5.7 -7 mg/l | 0.07-0.04 mg/l | 7.7-9 |

**Table (2):** Composition and proximate analysis of the experimental diets for Nile tilapia (*Oreochromis niloticu*s) fingerlings fed diets supplemented with different levels of Duckweed from diet protein.

| Ingredients (Protein %) | Experimental Diets | | | | |
|---|---|---|---|---|---|
| | **T1(Control)** | **T2 (25%)** | **T3 (50%)** | **T4 (75%)** | **T5 (100%)** |
| **Fish meal (70%)** | 40 | 40 | 40 | 40 | 40 |
| **Duckweedmeal (38%)** | 0 | 112.5 | 225 | 337.5 | 450 |
| **Soybean meal (44%)** | 450 | 337.5 | 225 | 112.5 | 0 |
| **Corn gluten (60%)** | 70 | 70 | 70 | 70 | 70 |
| **Rice bran (12%)** | 100 | 100 | 100 | 100 | 100 |
| **Yellow corn (8%)** | 207 | 207 | 207 | 207 | 207 |
| **Wheat bran (13%)** | 100 | 100 | 100 | 100 | 100 |
| **Soybean oil** | 30 | 30 | 30 | 30 | 30 |
| **DCP[1]** | 1 | 1 | 1 | 1 | 1 |
| **Vitamin& Mineral Mix[2]** | 2 | 2 | 2 | 2 | 2 |
| **TOTAL** | 1000 | 1000 | 1000 | 1000 | 1000 |
| **Chemical Composition (%)** | | | | | |
| **Dry matter** | 89.95 | 89.94 | 70.69 | 61.06 | 50.31 |
| **Crude protein** | 30.36 | 29.9 | 29.4 | 30.1 | 30.00 |
| **Ether extract** | 7.65 | 8.42 | 8.8 | 10.10 | 10.75 |
| **Crude fiber** | 6.52 | 5.7 | 6.1 | 6.8 | 7.1 |
| **Ash** | 5.32 | 6.76 | 7.20 | 8.61 | 8.9 |
| **N.F.E[3]** | 51.15 | 49.22 | 48.5 | 44.39 | 43.25 |
| **Gross energy Kcal/ 100g[4]** | 444.9 | 445.4 | 443.5 | 442.77 | 443.6 |
| **P/E ratio CP/GE kcal[5] mg/kcal** | 66.13 | 67.13 | 66.29 | 67.98 | 67.92 |

1) DCP (Di calcium phosphate)
2) Each Kg vitamin & mineral mixture premix contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin,20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.
3) Nitrogen Free Extract = 100 – (%Protein + %Fat + %Fiber + %Ash).
4) Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11Kcal/g). According to (NRC, 2011).
5) Protein Energy Ratio. ( crud protein /GE Kcal ). According to (NRC, 2011)

**Experimental Methodology**

**Fish samples**

At the start and the end of the main experimental period (56 day), 5 fish were randomly taken from each experimental group. Fish were used for chemical analysis of the whole body. The tested diets and body were analyzed for crud protein (CP %) ether extract (EE %), crude fiber (CF %), ash (%) and moisture. The whole body composition of fish samples were analyzed except crud fiber (CF %) according to the procedures described by standard (A O A C). The nitrogen free-extract (NFE %) was calculated by differences.

**Growth performance parameters**

The growth performance and feed utilization parameters are calculated according to the follow:

Average Weight Gain (AWG)

(AWG) = Average final weight (g) – Average initial weight (g)

Average Daily Gain (ADG)

(ADG) = [Average final weight (g) – Average initial weight (g)] / time (days)

Specific Growth Rate (SGR %/day)

(SGR % / day) = 100 [Ln Wt1 – Ln Wt. 0 / t]

***Where***: - Ln: normal log, Wt. 0: initial weight (g) Wt. 1: final weight (g) T: time of days

**Feed and protein utilization parameters**

Feed and protein utilization parameters are calculated according to the following equations:

Conversion Ratio (FCR)

FCR = Total feed consumption/weight gain.

Feed Efficiency (FE) = weight gain/Total feed consumption

Protein Efficiency Ratio (PER) = weight gain/protein consumed

Survival (%):

SR = Ni × 100/N0

***Where***: Nt = Total number of fish survived in tank at end of experiment

N0 = Total number of fish survived in tank at beginning of experiment

**Statistical analysis**

The data obtained in this study were analyzed by one-way ANOVA procedure of Statistical Analysis System (SAS Institute, 1998). Means were compared by Duncan's new multiple ranges test (Duncan, 1955).

***Where*: Yij = μ + Di + eij**

Yij = the observation of the j th individual from D th Diet

μ = the overall mean.

Di = The Fixed effect of the D th Diet

eij = The random error associated with the individual j

**Economical evaluation**

The cost of feed to raise unit biomass of fish was estimated by a simple economic analysis. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study.

Cost/kg diet (LE) = Cost per Kg diet L.E

Consumed feed to produce 1kg fish (kg) = Feed intake per fish per period/ final weight per fish Kg/Kg

Feed cost per kg fresh fish (LE) = Step 1 × step 2

Relative % of feed cost/ kg fish = Respective figures for step 3/highest figure in this step

Feed cost/1Kg gain (LE) = Feed intake per Kg gain × step 1

Relative % of feed cost of Kg gain = Respective figures for step 5/highest figure in this step

## RESULTS AND DISCUSSION

**Growth Performance**

The effects of Duckweed supplementation on growth performance and feed utilization of monosex Nile Tilapia (*Oreochromis niloticus*) fingerlings fed tested diets is presented in Table (3). No significant differences (P<0.05) in initial body weight were found among the different experimental treatments, indicating the accuracy of randomization process between the experimental treatments. It was observed that groups of fish fed the diet containing Duckweed 25 % showed highest values of body weight, weight gain, relative growth rate (RGR.), specific growth (SGR) and the lowest body weight, weight gain, relative growth rate (RGR.), specific growth were obtained in group of fish on T4 and T5 (75 and 100% replacement of soybean meal protein with duckweed meal. In agreement with Fasakin *et al.* (1999). They stated that increase in dietary duckweed inclusion resulted in progressively reduced growth performance and nutrients utilization of fish. Ahamad *et al*. (2003) reported also the replacement of sesame oil cake by duckweed in broiler diet. They stated that partial replacement of the costly oil seed by cheaper unconventional duckweed in broiler diet resulted in increased profitability. The protein efficiency ratio in the diets at 10 to 20% inclusive levels of duckweed meal in the experimental diet showed no significant difference compared with the control diet. Close results was obtained by Bairagi *et al*. (2002) reported that 30% fermented *lemna* leaf meal incorporated in the diet of *Labeo rohita* gave the best performance in terms of growth response, food conversion ratio and protein efficiency. Yilmaz *et al*. (2005) reported no significant difference between the growth performance of fish that were fed diets containing up to 20% duckweed and fish that were fed the control diet.

Feed intake (FI) and Protein Efficiency Ratio (PER) was significantly highest (P<0.05) in 25% replacement of soybean protein with duckweed protein (Table 3). In agreement with Fasakin *et al*. (1999) they reported that there was no significant difference in (P<0.05) in nutrient utilization of fish fed on diets containing up to 20% duckweed inclusion and the control. They however, stated that increase in dietary duckweed inclusion resulted in progressively reduced growth performance and nutrients utilization of fish. This report is similar to the findings of this study.

Inclusion of duckweed meal in the diet of other animals to replace fishmeal or soybean has also been reported by Samnang (1999), and Becerra *et al.* (1995) and Yilmaz *et al*. (2005). At the end of the experiment the group of fish on T1, T2 had a significantly highest (P<0.05) PER and FI than the rest of experimental groups T3, T4 and T5. The lowest FCR was obtained in group of fish on T1 and T2 and the highest FCR was found in group of fish on T4 and T5. In agreement with Becerra *et al.* (1995) and Samnang (1999), Moreover, Bairagi *et al.* (2002) reported that 30% fermented lemna leaf meal incorporated in the diet of *Labeo rohita* gave the best performance in terms of feed conversion ratio and protein efficiency. In agreement with Bairagi *et al.* (2002) reported that 30% fermented lemna leaf meal incorporated in the diet of *Labeo rohita* gave the best performance in terms of growth response, food conversion ratio and protein efficiency.

**Table (3):** Effect of replacement of soybean meal with duckweed on growth performance and feed utilization of fingerlings (g Mean ± SE) of monosex Nile tilapia (*O. niloticus*) throughout the experimental period (56 days)

| Items | Duckweed levels (%) | | | | |
|---|---|---|---|---|---|
| | Control T (1) | T (2) | T (3) | T (4) | T (5) |
| **Average Initial weight (g)** | 4. 1±0.1 | 4 .20 $^{a}$±0.01 | 4 .2±0.01 | 4 .11±0.01 | 4 .20±0.14 |
| **Average Final weight (g).** | 28.70 $^{a}$±0.08 | 28.50 $^{a}$±0.7 | 22 $^{b}$±0. 3 | 19.21 $^{c}$±0.7 | 17.41±0.4 |
| **Average Weight gain (g)** | 24.50 $^{a}$±0.13 | 24.30 $^{a}$±0.13 | 18.6 $^{b}$±0.13 | 16.69 $^{c}$±0.14 | 14.1±0.14 |
| **Specific Growth Rate (SGR) (%/day)** | 3.48 $^{a}$±0.13 | 3.41 $^{a}$±0.13 | 2.95 $^{b}$±0.13 | 2.76 $^{c}$ ±0.14 | 2.54±0.14 |
| **Feed Intake (FI) (g)** | 47.99 $^{a}$±0.13 | 49.44 $^{a}$±0.13 | 43.3 $^{b}$±0.13 | 44.27 $^{c}$±0.14 | 41.14±0.14 |
| **Feed Conversion Ratio (FCR)** | 1.95 $^{a}$±0.13 | 2.03 $^{a}$±0.13 | 2.32 $^{b}$±0.13 | 2.7 $^{c}$±0.14 | 2.9±0.14 |
| **Protein efficiency Ratio (PER)** | 1.70 $^{a}$ ±0.13 | 1.60 $^{a}$ ±0.13 | 1.40 $^{b}$ ±0.13 | 1.20 $^{c}$ ±0.14 | 1.10±0.14 |
| **Feed Efficiency (FE)** | 0.51 $^{a}$ ±0.01 | 0.49 $^{a}$ ±0.01 | 0.43 $^{b}$ ±0.01 | 0.38 $^{c}$ ±0.01 | 0.34±0.14 |
| **Survival Rate (%)** | 97 $^{a}$ ±0.13 | 97 $^{a}$ ±0.13 | 93 $^{c}$ ±0.13 | 94 $^{b}$ ±0.13 | 93 $^{c}$ ±0.13 |

Means ± SE in the same letter in the same row is not significantly different at P<0.05.

**Carcass composition of fish**

Table (4) shows the effect of replacement of soybean meal protein by duckweed at different levels on body composition for 56 days. Duckweed was significantly (P<0.05) affected whole-fish body composition (Table 4). Fish fed with the control diet had the lowest protein content; however, all diets contained with duckweed appeared to improved protein content. Carcass lipid and protein content was also affected by dietary treatments with the highest values in (T1) and (T2), which were statistically (P<0.05) highest than the rest of dietary treatments. On the other hand, changes in protein and lipids contents in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rat (Yilmaz *et al*., 2005).

**Table (4):** Effect of replacement of soybean meal protein with duckweed on body composition of monosex Nile tilapia (*O. niloticus*) fingerlings on dry matter basis

| Chemical composition | Dry matter (%) | Crude protein (%) | Ether extract (% ) | Ash (%) |
|---|---|---|---|---|
| **Initial** | 73.40±0.27 | 61.20±0.27 | 22.80±0.27 | 12.60±0.27 |
| **Control T1** | 72.50±0.27 | 63.70±0.41 $^{a}$ | 23.80±0.18 $^{a}$ | 12.50±0.13 $^{a}$ |
| **T2** | 72.50±0.22 | 64.80±0.51 $^{a}$ | 24.70±0.12 $^{a}$ | 12.90±0.23 $^{a}$ |
| **T3** | 72.50±0.25 | 63.30±0.21 $^{b}$ | 22.80±0.14 $^{b}$ | 12.10±0.22 $^{a}$ |
| **T4** | 72.50±0.22 | 62.60±0.40 $^{c}$ | 22.10±0.16 $^{c}$ | 12.10±0.20$^{a}$ |
| **T5** | 72.50±0.24 | 62.20±0.32 $^{c}$ | 25.10±0.18 c | 12.40±0.22a |

**Note:** Values are means ± SE of three replications. Means in the same column having different superscripts are significantly different (P <0.05).

**Economical evaluation**

Calculations of economic efficiency of the tested diets based on the cost of feed, costs of one Kg gain in weight and its ratio with the control group are shown in Table 5. The highest Feed cost /1Kg fresh fish gain (3.25 LE) for T1 followed by 2.94 L.E for T2, 2.14 LE for T3. 1.92 L.E for T4 and 1.44L.E for T5 respectively.

Feed cost / Kg gain were 13.20, 12.08, 11.48, 11.72 and 10.12 For T1, T2, T4, T4 and T5 respectively and Relative % of feed cost of Kg gain (87%) in group of fish fed diet 2 (25% Duck weeds).This indicates that T5 was the most economic than the rest of experimental groups. These results indicates that replacement of soybean meal protein with duckweed at level 100% were the most economic in term of economical point of view. Moreover 25% replacement soybean meal protein with duckweed was the best in terms of nutritional point of view.

## CONCLUSION

In conclusion, it would be advisable to include up to 25% duckweed in commercial feed for (*O. niloticus*) fingerlings in order to produce results similar to those obtained with the control diet with respect to growth, feed utilization, and body composition. These findings also support the use of duckweed in commercial grow-out carp feeds as a dry ingredient. Duckweed meal should be considered for both cold and warm water fish nutrition.

**Table (5):** Economic analysis of Nile tilapia fingerlings (*O. niloticus*) fed different levels of Duckweed for 56 gains (L.E)

| Components | Treatments | | | | |
|---|---|---|---|---|---|
| | Control T(1) | T (2) | T (3) | T (4) | T (5) |
| **Cost/1 Kg/diet** | 6.77 | 5.95 | 4.95 | 4.34 | 3.49 |
| **(FI)2** | 47.99 | 49.44 | 43.3 | 44.27 | 41.14 |
| **Feed cost per kg fresh fish (LE)3** | 3.25. | 2.94. | 2.14 | 1.92 | 1.438 |
| **Relative % of feed cost/kg fish4** | 100 | 90.46 | 65.84 | 59.07 | 44.24 |
| **(FCR)5** | 1.95 | 2.03 | 2.32 | 2.70 | 2.90 |
| **Feed cost/1Kg gain (LE6** | 13.20 | 12.08 | 11.48 | 11.72 | 10.12 |

1- Cost/Kg/diet
2- Feed Intake
3- Feed cost per/ kg /fresh fish (LE) = Cost/Kg/diet **x**(FI) **/** 100
4- Relative % of feed cost/ kg fish
5- Feed Conversion Ratio
6- Feed cost /1Kg gain (LE) = (FCR) x Cost.

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# تأثير إحلال كسب فول الصويا بمسحوق عدس الماء علي أداء النمو والإستفاده الغذائية لإصبعيات سمك البلطي النيلي

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أجريت هذه الدراسة بمفرخ السلام – القنطرة شرق محافظة الإسماعيلية - بهدف دراسة تأثير استبدال كسب فول الصويا بنبات عدس الماء بنسب مختلفة من بروتين العليقة وذلك على أداء النمو، الاستفادة الغذائية، مكونات الجسم، والتقييم الاقتصادي وذلك علي اصبعيات سمك البلطي النيلي استخدم فيها أوزان 4.1±0.1 جرام وكانت نسب الإحلال كالتالي (٢٥، ٥٠، ٧٥ و ١٠٠% بالاضافه إلي العليقه الكنترول). وكانت أهم النتائج المتحصل عليها: ١) سجلت أفضل زيادة معنوية في الوزن النهائي، معدل النمو النوعي، الكفاءة التحويلية للغذاء، الكفاءة الغذائية، معدل استهلاك الغذاء، معامل الاستفادة من البروتين، وأعلي معدل حيوية في العليقة الثانية ذات نسبة إحلال (٢٥%) من كسب فول الصويا يليها العليقة ١، ٣، ٤ على التوالي. ٢) وجد أن العلائق التجريبية المستخدمة في التجربة أثرت معنويا على التحليل الكيماوي لمكونات الجسم (البروتين والدهن)، كما وجد إن اعلي نسبة لمحتوى البروتين والدهن كانت في العليقة رقم (٢) (٢٥%). كما لا يوجد تأثير معنوي للعلائق على محتوى الرطوبة والرماد في الجسم بين المعاملات التجريبية المختلفة. ٣) بدراسة تأثير المعاملات على الناحية الاقتصادية وجد إن أفضل معاملة هي العلية رقم (٢ ) (٢٥%) كنسبة إحلال من كسب فول الصويا بين المعاملات التجريبية المختلفة. ويستنتج من هذه الدراسة إن استبدال كسب فول الصويا بواسطة نبات عدس الماء بنسبة ٢٥% كان الأفضل من حيث مقاييس النمو، الاستفادة الغذائية، التحليل الكيماوي لمكونات الجسم والتحليل الاقتصادي في الظروف التجريبيه.